September 2022

Quick-Start Protocol

QIAcuity® OneStep Advanced Probe Kit

This protocol is optimized for the quantification of RNA and DNA targets using the QIAcuity OneStep Advanced Probe Kit with hydrolysis probes in a singleplex or multiplex (up to 5 targets) reaction using QIAGEN's QIAcuity instruments for digital PCR (dPCR).

The QIAcuity OneStep Advanced Probe Kit should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer and protected from light. Under these conditions, the components are stable for 12 months without showing any reduction in performance and quality, unless otherwise indicated on the label.

Further information

- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- *QIAcuity User Manual Extension*: **www.qiagen.com/HB-2839**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Refer to the *QIAcuity User Manual* and *QIAcuity User Manual Extension* for guidance on assay design and experimental setup for the QIAcuity platform.
- The QIAcuity OneStep Advanced Probe Kit has been specially formulated with a hot-start RT enzyme, allowing users to assemble reactions at room temperature, and to run up to four or eight plates in parallel on the QIAcuity Four or QIAcuity Eight instruments, respectively.

- The optional Enhancer GC is recommended for use with all Applied Biosystems™ TaqMan® assays, amplicons >150 bp in length, GC-rich amplicons, and RNA targets containing challenging secondary structures.
- The QuantiNova® Internal Control RNA (QN IC RNA) supplied with the kit can be used optionally as a reverse transcription and amplification control. To do so, users must separately purchase the QuantiNova IC Probe Assay (200) (cat. no. 205813), which can be detected in the yellow channel on QIAcuity instruments.

  Important: DO NOT purchase the QuantiNova IC Probe Assay Red 650 (500) (cat. no. 205824), as it is not optimized to work with QIAcuity.
- The QN IC RNA is detected as a 200 bp amplicon. The primer and probe sequences for the detection of the QN IC RNA have been bioinformatically validated for non-homology against hundreds of eukaryotic and prokaryotic organisms. Additionally, they have been experimentally tested against a multitude of human, mouse, and rat RNA samples from multiple tissues and cell lines.
- The QN Internal Control RNA comes at a concentration of ~1 x 10^5 to 1 x 10^6 copies/µL. Users are recommended to add the QN Internal Control RNA to the QIAcuity reactions at a final dilution of 1 to 1000 (e.g., create a working dilution by diluting the QN Internal Control RNA stock 1 to 10 in RNase-Free Water, which is then diluted 1 to 100 in the QIAcuity OneStep Advanced Probe reaction mix).

Procedure

Reaction mix setup

1. Place the 100x OneStep Advanced Reverse Transcription Mix on ice. Thaw the 4x QIAcuity OneStep Advanced Probe Master Mix, template RNA, primers, probes, Enhancer GC, and RNase-Free Water. Vigorously mix the QIAcuity OneStep Advanced Probe Master Mix and the individual solutions. Centrifuge the tubes briefly to settle the liquids.
2. Prepare a master mix according to Table 1 and the desired Nanoplate format.

Table 1. Preparing the QIAcuity OneStep Advanced Probe RT-dPCR reaction mix

Component	Volume/reaction		Final concentration
	Nanoplate 8.5k (24-well and 96-well)	Nanoplate 26k (24-well)	
4x OneStep Advanced Probe Master Mix	3 µL	10 µL	1x
100x OneStep Advanced RT Mix (Reverse Transcription)	0.12 µL	0.4 µL	1x
20x primer–probe mix 1*	0.6 µL	2 µL	0.4 µM forward primer 0.4 µM reverse primer 0.2 µM probe
20x primer–probe mix 2, 3, 4, 5* (for multiplex)	0.6 µL (each)	2 µL (each)	0.4 µM forward primer 0.4 µM reverse primer 0.2 µM probe
Enhancer GC† (optional)	1.5 µL	5 µL	–
RNase-Free Water	Variable	Variable	–
Template RNA (added at step 4)‡	Variable	Variable	–
Total reaction volume	**12 µL**	**40 µL**	

* For dye recommendations, see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension*.
† Enhancer GC is recommended for use with all Applied Biosystems TaqMan assays, amplicons >150 bp in length, GC-rich amplicons, and RNA targets containing challenging secondary structures.
‡ Appropriate template amount depends on various parameters.

3. Vortex the reaction mix well. Dispense appropriate volumes of the reaction mix into the wells of a standard 96-well PCR pre-plate.

   Note: The pre-plate may be assembled at room temperature.

4. Add template RNA to wells containing the reaction mix. Thoroughly mix the template RNA with the reaction mix by pipetting up and down.

One-step RT-dPCR protocol for all QIAcuity instruments

1. Transfer the contents of each well in the pre-plate to the wells of a Nanoplate.
2. Seal the Nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.
3. Place the Nanoplate into the QIAcuity instrument and start the RT-dPCR program.

Table 2. QIAcuity RT-dPCR cycling program

Step	Time	Temperature
Reverse Transcription	40 min	50°C
RT Enzyme Inactivation	2 min	95°C
2-step cycling (40 cycles)	–	–
Denaturation	5 s	95°C
Combined annealing/extension	30 s	60°C*

* Temperature during annealing/extension and number of cycles might vary depending on assay type.

Document Revision History

Date	Changes
April 2022	Corrected the step number referenced in Table 1
September 2022	Updated Notes before starting. Layout and editorial changes.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.